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Requester's Full Name: IT GITOWIKH Examiner #: 69630 Date: 5/4/01
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Title of Invention: _____

Inventors (please provide full names): _____

Earliest Priority Filing Date: _____

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5/30/01 considered

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Set	Items	Description
S1	1120320	(CELL? ? OR TISSUE? ?) (5N) CULTURE? ?
S2	19051	MM3 OR MM()3
S3	916	S1 AND S2
S4	2	S3 AND MECHANICAL
S5	494	AU=KORNBLITH P? OR AU=KORNBLITH, P?
S6	0	S5 AND S3
S7	667	S3 NOT PY>1996
S8	2	S7 AND MECHANICAL
S9	45	S7 AND ((TUMOR OR TUMOUR) (3N) VOLUME? ?)
S10	47	S7 AND ((TUMOR? ? OR TUMOUR? ?) (3N) VOLUME? ?)
S11	620	S7 NOT S10
S12	1719	CELLSMM3 OR (CELLS()MM3)
S13	589	S11 NOT S12
S14	571	S13 NOT ((100 OR 200) ()MM3)
S15	558	S14 NOT (PER()MM3)
S16	545	S15 NOT ((TUMOR? ? OR TUMOUR? ?) (3N) SIZE? ?)
S17	513	S16 NOT PY>1995
S18	22	S17 AND PIECE? ?
S19	19	RD S18 (unique items)
S20	23	S17 AND (MINC? OR CUT OR CUTTING)
S21	16	RD S20 (unique items)
S22	26	S8 OR S19 OR S21
S23	24	S22 NOT (CLEAR()CUT)

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23/7/1 (Item 1 from file:155)

DIALOG(R) File 155:MEDLINE(R)

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08703400 96095294

Expression of epidermal growth factor (EGF) and the EGF receptor in the porcine oviduct.

Swanchara KW; Henricks DM; Birrenkott GP; Bodine AB; Richardson ME
Animal, Dairy, and Veterinary Sciences Department, Clemson University,
South Carolina 29634-0361, USA.

Biology of reproduction (UNITED STATES) Oct 1995, 53 (4) p911-22,
ISSN 0006-3363 Journal Code: A3W

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The production, secretion, and localization of epidermal growth factor (EGF) and the distribution of the EGF receptor (EGF-R) were examined in the

isthmus (I) and ampulla (A) of the oviducts from cyclic (C) and early-pregnant (P) gilts. Sexually mature gilts (n = 20) were divided equally into two groups: C and P. P gilts were bred twice (at 0 and 24 h), and all gilts were killed 48 h after onset of estrus. After removal of reproductive tracts, oviducts were isolated, flushed, opened longitudinally, divided by anatomical region, cut into 1-3-mm³ pieces, and placed in Dulbecco's modified Eagle's Essential medium (DMEM: F-12 + ITS [insulin, 5 micrograms/ml; transferrin, 5 micrograms/ml; and selenious acid, 5 ng/ml] + antibiotic). Half the tissue and medium were immediately homogenized and centrifuged, and the supernatant was removed. The remaining tissue was cultured in the medium for 24 h at 37 degrees C and 5% CO₂, then prepared similarly for analysis. EGF was measured in the supernatant by a heterologous RIA. Concentration of EGF was expressed as nanogram/milliliter of EGF per milligram of protein in wet tissue. EGF concentrations were present in both regions of the oviducts of C and P gilts. It was greater in I than in A tissues for both C (I = 16.21 ng/ml vs. A = 13.91 ng/ml; p < 0.05) and P gilts (I = 14.27 ng/ml vs. A = 12.53 ng/ml; p < 0.10). Higher concentrations of EGF were found in I tissue of C gilts than in P gilts (C = 16.21 ng/ml vs. P = 14.27 ng/ml; p < 0.05). The media assayed from cultured explants of I and A sections from C and P gilts gave results that were highly correlated with those of immediately prepared tissue sections. Localization of EGF in frozen oviductal tissue sections was demonstrated by immunohistochemistry. The primary site of EGF immunostaining occurred in the epithelial cells (with highest intensity at the apical border) of both C and P gilts. A and I tissue sections from C gilts showed localization of EGF immunostaining mainly in epithelial cells and lamina propria cells, while those from P gilts stained less intensely. The presence of EGF-R was shown by incubating tissue imprints and frozen sections with EGF-erythrosin isothiocyanate, which revealed that EGF-R were distributed mainly on the membranes of epithelial cells. The study indicates that EGF and EGF-R are present in oviductal epithelial cells in both C and P gilts, with the highest concentration of EGF in C gilts. (ABSTRACT TRUNCATED AT 250 WORDS)

23/7/2 (Item 2 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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8/30 | 08684563 95139736

A tissue - culture model for the study of canine vocal fold fibroblasts.

Broadley C; Gonzalez DA; Nair R; Koriwchak MJ; Ossoff RH; Davidson JM
Department of Otolaryngology, Vanderbilt University Medical Center,
Nashville, TN 37232.

Laryngoscope (UNITED STATES) Jan 1995, 105 (1) p23-7, ISSN 0023-852X
Journal Code: L1W

Languages: ENGLISH

Document type: JOURNAL ARTICLE

A tissue - culture model has been developed for the study of fibroblasts from the canine vocal fold. Laryngeal tissue (lamina propria) obtained from euthanized dogs is rinsed, cut into 1-mm³ pieces, and incubated in 5% carbon dioxide at 37 degrees C. A confluent monolayer is established within several days. Detectable levels of elastin in the tissue culture supernatant are measured by an indirect enzyme-linked immunosorbent assay. Various external agents have been shown to affect elastin production. The effects of KTP laser irradiation, hydrocortisone

(1.3 $\mu\text{mol/L}$), transforming growth factor-beta (10 ng/mL), and human leukocyte elastase have been measured. Thus the canine vocal fold fibroblast tissue culture is established as a model for further investigations to improve wound healing and to understand the wound-healing process following laryngeal microsurgery.

23/7/3 (Item 3 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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08513021 96192961

Bovine oocyte diameter in relation to maturational competence and transcriptional activity.

Fair T; Hyttel P; Greve T

Department of Reproduction, Royal Veterinary and Agricultural University, Frederiksberg, Denmark.

Molecular reproduction and development (UNITED STATES) Dec 1995, 42
(4) p437-42, ISSN 1040-452X Journal Code: AN7

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The aims of the present series of experiments were to establish a possible relationship between bovine oocyte diameter and follicle size, investigate the developmental ability of oocytes of different diameter groups, and investigate the relationship between oocyte diameter and RNA transcriptional activity of the oocyte. Follicles were recovered from slaughterhouse ovaries by mechanical dissection, measured, and assigned to one of the following size categories: ≥ 4 mm, $< 3-4$ mm, $< 2-3$ mm, $< 1-2$ mm, and < 1 mm. Oocytes were liberated from the follicles and their diameters recorded. The correlation coefficient between oocyte diameter and follicle size was 0.32 ($P < 0.0001$). Oocytes were recovered by aspiration and/or slicing of slaughterhouse ovaries and divided into four groups (< 100 microns, $100- < 110$ microns, $110- < 120$ microns, and > 120 microns) based on diameter. Oocytes were processed through standard procedures for in vitro maturation and stained in order to assess nuclear development. Rates of in vitro development to metaphase II were 21.2%, 42.3%, 75.9%, and 80.7%, respectively, for the four groups. On a separate occasion immature oocytes from the above diameter groups were cultured in the presence of 3H-uridine for 45 min and scored for degree of RNA synthesis as indicated by the presence of autoradiographic labeling. Oocytes < 110 microns showed a greater degree of 3H-uridine incorporation than those ≥ 110 microns, suggesting that they were involved in RNA synthesis and therefore still in the growth phase.

23/7/4 (Item 4 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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08273772 95236262

A simple method for the propagation of cervical lymphocytes.

Moscicki AB; Hunter SD; Garland S; Quinn M; Crowe SM; Shortman K; Stites D

Department of Pediatrics, University of California, San Francisco.

Clinical and diagnostic laboratory immunology (UNITED STATES) Jan 1995, 2 (1) p40-3, ISSN 1071-412X Journal Code: CB7

Contract/Grant No.: R01CA51323, CA, NCI; P01A121912

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Local immune function is most likely a key influence in the establishment of human papillomavirus infections and its subsequent disease. Unfortunately, little information is known about local cervical immunity, and even less is known about human papillomavirus immunoreactivity. In addition, studies of local immunoreactivity have been hampered by the technical difficulty in obtaining cervical lymphocytes. The objective of the present study was to develop a simple method for the propagation of cervical lymphocytes from biopsy-size specimens. Cervical tissue was obtained from women undergoing a hysterectomy. Cervical samples measuring approximately 3 by 5 by 2 mm were minced and divided into two portions. One portion was digested by standard digestion methods and density gradient lymphocyte separation. The sample was then immunocharacterized for CD4 and CD8 cells by flow cytometry. The other portion was minced into 1-mm³ sections, and each section was placed into a separate well with tissue culture medium and interleukin-2. Lymphocyte counts and immunophenotypic analysis were performed after 18 to 20 days in culture. After 18 to 20 days in culture, the analysis demonstrated that this method of direct lymphocyte culture from a biopsy specimen yielded approximately 1×10^6 to 5×10^6 lymphocytes. Immunophenotypic studies of the digested sample at day 0 revealed CD4-to-CD8 ratios of between 0.7:1 and 3.5:1, and at days 18 to 20 they revealed ratios of between 2.3:1 and 98:1. In summary, we developed a simple technique for propagating cervical lymphocytes from small tissue samples for the study of the local immune response. Studies are under way to optimize lymphocyte growth and to preserve CD8 populations.

23/7/5 (Item 5 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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08068457 95079096

Immature mouse uterine tissue in organ culture: estrogen-induced growth, morphology and biochemical parameters.

Newbold RR; Hanson RB; Jefferson WN

Developmental Endocrinology and Pharmacology Section, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina 27709.

In vitro cellular & developmental biology (UNITED STATES) Aug 1994, 30A (8) p519-28, ISSN 1071-2690 Journal Code: BZE

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Although estrogens have been shown to stimulate a variety of morphologic and biochemical changes in the uterus in vivo, no clear consistent demonstration of similar responses in vitro have been made; thus, a defined organ culture system using the immature mouse uterus was established to study the possibility of demonstrating estrogenic responses in vitro. Uterine tissue from immature outbred mice (17 to 24 days of age) were cut crosswise in 1-mm³ coins and cultured in a defined medium in the absence of serum, phenol red, or growth factor supplements. Diethylstilbestrol (DES), a synthetic estrogen, was added to the media at doses ranging from 1 to 100 ng/ml. The effect of DES on uterine cell proliferation was assessed by morphologic changes in uterine epithelial and stromal cells, increase in number of epithelial cells per unit basement membrane, increase in height of luminal epithelial cells, and [3H]thymidine incorporation. Functional changes were determined by measuring the amounts of the estrogen-inducible

uterine protein, lactoferrin, that was localized in the epithelial cells and secreted into the media, and the localization of the estrogen receptor in the cultured tissues. Results indicate that under the described conditions of culture, estrogens like DES can induce morphologic and biochemical responses in the uterus that are similar to those seen in vivo. This organ culture system will aid in the investigation of various mechanisms involved in the hormonal regulation of growth and differentiation of estrogen target tissues.

23/7/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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07716445 94125473
Cryopreservation and culture of the human fetal brain tissues.
Cai RS; Xue DL; Jiang XH
Department of Neurosurgery, Tongji Hospital, Tongji Medical University, Wuhan.
Journal of Tongji Medical University (CHINA) 1993, 13 (3) p138-42,
ISSN 0257-716X Journal Code: KAJ
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Human embryos after 3-4.5 months of gestation were obtained with abortion. The brain tissue of the bodies was scissored up to obtain 1-3 mm³ pieces, and 7% dimethyl sulfoxide (DMSO), as a cryoprotectant, was added, and then stored at -70 degrees C for 1-30 days or at -196 degrees C for 1-84 days. The survival rate of stored cells was 64%-88%. During 6 days of storage with neuron culture medium, the survival rate of cells at 4 degrees C is over 50% each day, but, as time goes on, the count of the cells is getting less and less. The cells washed out DMSO after cryopreservation and the planting fresh cells can adhere to the wall of the culture bottle, grow, display various forms of neurons and gliocytes. From the above findings, it was suggested that: 1) The fetal human brain tissue, handled properly, can endure cryopreservation with 7% DMSO as a cryoprotective agent; 2) The storage time was related insignificantly to the survival rate of the tissues stored; 3) It is available for a short preservation at 4 degrees C; and 4) It is possible to set up a bank of fetal human brain tissue.

23/7/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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07701152 94096475
Evaluation of CSF variables as a diagnostic test for bacterial meningitis.
Deivanayagam N; Ashok TP; Nedunchelian K; Ahamed SS; Mala N
Clinical Epidemiology Unit, Madras Medical College, India.
Journal of tropical pediatrics (ENGLAND) Oct 1993, 39 (5) p284-7,
ISSN 0142-6338 Journal Code: KAW
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Specific aetiological diagnosis of bacterial meningitis (BM) in developing countries is often difficult. Frequently, differentiating BM from viral and TB meningitis is not easy. A study was carried out with the

easily and quickly performed CSF morphological and biochemical changes as a diagnostic test against the gold standard of CSF culture and/or the latex agglutination test (LAT). Children between 2 months and 11 years of age, suspected to have acute meningitis, were prospectively recruited. CSF cell count and morphology, Gram stain, culture, and protein and sugar estimations were carried out as per standard procedures. The laboratory personnel were blind to the clinical details and the findings of each other. Diagnosis based on gold standard was possible in 55 out of 114 cases. With CSF polymorphs > 60 per cent and sugar < 50 per cent of blood level as constants, various levels of total cells and protein were considered for their diagnostic properties. The protein level was not useful. We found the best cut-off level of cell count for diagnosis of BM to be 300/mm³, based on the receiver operating characteristics curve, the point of maximum accuracy. These findings were validated by comparing the clinical features, CSF changes and outcome characteristics of non-confirmed cases with the above criteria with the confirmed cases; these were found to be the same except for age. (ABSTRACT TRUNCATED AT 250 WORDS)

23/7/8 (Item 8 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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07338755 90242303

Effect of steroidal and nonsteroidal antiestrogens on the growth of a tamoxifen-stimulated human endometrial carcinoma (EnCa101) in athymic mice.

Gottardis MM; Ricchio ME; Satyaswaroop PG; Jordan VC

Department of Human Oncology, University of Wisconsin Clinical Cancer Center, Madison.

Cancer research (UNITED STATES) Jun 1 1990, 50 (11) p3189-92, ISSN 0008-5472 Journal Code: CNF

Contract/Grant No.: P01-CA20432, CA, NCI; T32-CA09471

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Tamoxifen (TAM), a nonsteroidal antiestrogen, is used in the adjuvant treatment of breast cancer. Previous studies, however, have indicated that some human breast and endometrial tumors are stimulated to grow with TAM in the athymic mouse. One such TAM-stimulated tumor is the EnCa101 human endometrial adenocarcinoma. Our aim was to evaluate the ability of different doses of TAM or other nonsteroidal antiestrogens to stimulate the growth of EnCa101 tumors in athymic mice. Additionally we have evaluated less estrogenic antiestrogens (two steroidal antiestrogens, RU 39,411 and ICI 164,384, and two nonsteroidal antiestrogens, keoxifene and MER-25) for their ability to inhibit TAM-stimulated growth. All experiments were done in ovariectomized athymic mice transplanted in the axillary mammary fat with 1-mm³ pieces of EnCa101 tumor. Sustained release preparations (0.5-2.0-cm Silastic capsule or 5-mg TAM cholesterol pellet) of TAM caused similar tumor growth. The growth rate was not altered by an additional daily i.p. injection of 1 mg TAM in 0.1 ml peanut oil. A 3-mg TAM daily dose was toxic. Four weeks of treatment (100-micrograms s.c. injections, every other day) with nonsteroidal antiestrogens, trioxifene mesylate, enclomiphene, or nafoxidine stimulated tumor growth. However, keoxifene stimulated this tumor to a lesser degree than TAM and partially inhibited TAM-stimulated growth. ICI 164,384 showed no stimulatory activity (1-mg s.c. injections every other day) alone compared to controls but inhibited TAM-stimulated (0.25-cm Silastic capsule) growth. In a parallel experiment, RU 39,411 (1-mg s.c. injections every other day) stimulated EnCa101 to

grow. In contrast when RU 39,411 was administered in a sustained release preparation (2.0-cm Silastic capsule) there was no stimulatory growth compared to controls. Additionally RU 39,411 inhibited TAM-stimulated growth, but the low-potency antiestrogen, MER-25, was less effective in this regard. These data suggest that less "estrogenic" antiestrogens can inhibit TAM-stimulated tumor growth in vivo. Thus these compounds or derivatives may prove useful as a second-line endocrine therapy should TAM-stimulated tumor growth occur in the clinic.

23/7/9 (Item 9 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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07193814 93111386

5/30
A human endometrial explant system: validation and potential applications.

Dudley DJ; Hatasaka HH; Branch DW; Hammond E; Mitchell MD
Department of Obstetrics and Gynecology, University of Utah School of Medicine, Salt Lake City 84132.

American journal of obstetrics and gynecology (UNITED STATES) Dec 1992, 167 (6) p1774-80, ISSN 0002-9378 Journal Code: 3NI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

OBJECTIVE: Our objective was to describe an in vitro explant system to study the regulation of prostaglandin production by human endometrium. STUDY DESIGN: Segments of late-luteal-phase endometrium were obtained aseptically at the time of endometrial sampling. The endometrium was cut into 1 mm³ pieces and applied to the polycarbonate membrane of tissue - culture -well inserts for 12-well plates (Costar Transwell cell culture chamber inserts, 0.4 microns pore size). After placing the well inserts, culture medium was carefully applied. The explants were incubated at 37 degrees C in 5% carbon dioxide in air, and the culture medium was changed daily. RESULTS: Electron microscopic examination and lactate dehydrogenase determinations of the explants revealed cellular viability for < or = 5 days of culture. Endometrial explants responded to treatment with interleukin-1 beta and tumor necrosis factor by a concentration-dependent increase in the production of prostaglandin E2. Costimulation of late luteal endometrial explants with interleukin-1 beta (10 ng/ml) and progesterone (10(-6) mol/L) resulted in variable production of prostaglandin E2, suggesting that the histologic examination of the endometrium does not necessarily reflect the functional properties of the endometrium. CONCLUSIONS: Our data show that when used with human endometrial tissue this explant system maintains tissue viability and responsiveness for < or = 5 days. This approach to explant methods is simple and provides a flexible model to study the regulation of the production of bioactive substances by human endometrial tissue.

23/7/10 (Item 10 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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05778772 92033121

[Establishment of acinar cells from human salivary gland tissue by transformation of SV40]

Kiyonobu S

Shika Kiso Igakkai zasshi (JAPAN) Jun 1989, 31 (3) p248-56, ISSN 0385-0137 Journal Code: A9T

Languages: JAPANESE Summary Languages: ENGLISH

Document type: JOURNAL ARTICLE ; English Abstract

A piece of minor salivary gland tissue obtained from the lip of a 16 years old female was minced to about 3 mm³ by fine pincettes and , cultured with 10% FCS containing MEM supplemented with EGF (10 ng/ml), fungizon (10 mcg/ml) and kanamycin (60 mcg/ml) in a 5% CO₂ incubator. Many small bubbles of saliva were found on the surface of the fragments after 3 to 4 days of incubation and outgrowth of cells from the fragments was observed from 7 days of incubation. Monolayer cells of the outgrowth were trypsinized and passaged with fresh culture medium. At the 8th passage, monolayer cells were infected with SV40 at moi 100PFU/cell. After 18 hour-incubation, the suspension of the infected cells was incubated at densities of 10(4) and 10(3) cells/dish within 0.33% agar containing culture medium. Transformed colonies were picked up from soft agar medium and 3 of the 28 colonies were identified as being acinar cells of the salivary gland, since secretory granules and mucosubstances were specifically proved in the cytoplasm of these cells after 2 to 4 days of incubation. One of the typical clone cells was named HA-16 cells. However, the appearances of the secretory granules and mucins in the cytoplasm of the HA-16 cells depended on the cellular growth cycle, i.e. secretory granules and mucins were not found in the growing cells (G1-S-G2-M phase) but many secretory granules and mucins could be recognized in the non-dividing cells (G0 phase). (ABSTRACT TRUNCATED AT 250 WORDS)

23/7/11 (Item 11 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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05559259 89336685

Differential ability of antiestrogens to stimulate breast cancer cell (MCF-7) growth in vivo and in vitro.

Gottardis MM; Wagner RJ; Borden EC; Jordan VC

Department of Human Oncology, University of Wisconsin Clinical Cancer Center, Madison 53792.

Cancer research (UNITED STATES) Sep 1 1989, 49 (17) p4765-9, ISSN 0008-5472 Journal Code: CNF

Contract/Grant No.: CA20432, CA, NCI; T32-CA09471, CA, NCI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

We have previously described an MCF-7 breast cancer cell variant, MCF-7TAM, which is stimulated to grow in athymic mice by tamoxifen (TAM) (M. M. Gottardis and V. C. Jordan, Cancer Res., 48:5183-5187, 1988). Earlier experiments have shown that TAM exhibits some profound estrogen-like effects in mice whereas TAM is less estrogenic in the rat. The aim in these studies was to compare the ability of MCF-7TAM to grow in different host environments and to determine whether the TAM-stimulated phenotype could be maintained in vitro. Ovariectomized athymic mice and rats were implanted with 1-mm³ pieces of MCF-7TAM tumor and treated with estradiol, TAM, or control silastic capsules. After 9 weeks of growth in either species, TAM or estradiol-treated groups both had sustained growth of MCF-7TAM compared with the control groups. To determine the effects of estradiol and TAM on immune function in athymic mice, splenocytes from treated or control athymic mice, challenged with poly(I:C), were assayed for natural killer (NK) cell activity against

51Cr-labeled YAC1 target cells. Both estradiol and TAM abolished lytic activity by 12 weeks of treatment. To evaluate the role of a decrease in NK-cell activity in the host on growth of MCF-7TAM xenografts we compared the growth effects in athymic and NK-cell deficient, ovariectomized beige mice. TAM stimulated MCF-7TAM in both beige and athymic mice; however, the tumor grew more rapidly in control beige mice than in control athymic mice. This study demonstrated that TAM-stimulated growth could occur in vivo. However, TAM or 4-hydroxytamoxifen did not cause a stimulation of MCF-7TAM compared with wild-type MCF-7 cells when experiments were conducted in vitro. These studies demonstrate that a suppression immune function can facilitate the growth of MCF-7TAM in athymic animals. However, additional components of the host environment contribute to TAM-stimulated growth in vivo.

23/7/12 (Item 12 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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05515064 89213275

Adhesive, invasive, and growth properties of selected metastatic variants of a murine large-cell lymphoma.

Nicolson GL; Belloni PN; Tressler RJ; Dulski K; Inoue T; Cavanaugh PG
Department of Tumor Biology, University of Texas M.D. Anderson Cancer Center, Houston.

Invasion & metastasis (SWITZERLAND) 1989, 9 (2) p102-16, ISSN 0251-1789 Journal Code: GV4

Contract/Grant No.: R35-CA44352, CA, NCI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The adhesive, invasive, and growth properties of parental murine large-cell lymphoma cells of low metastatic potential (RAW117-P) were compared to in-vivo-selected sublines of high metastatic potential to liver (RAW117-H10) or lung (RAW117-L17). Using small (approximately 0.5 mm³) pieces of syngeneic organ tissue (lung, liver, kidney) we found that RAW117-L17 cells selectively attached to and invaded lung tissue, whereas RAW117-H10 cells preferentially attached to and invaded liver tissue. We measured adhesion to microvessel endothelial cells established from syngeneic lung and liver and found that the RAW117-L17 cells bound to lung microvessel endothelial cells at significantly higher rates than the other lines, and RAW117-H10 and -L17 cells attached to hepatic sinusoidal endothelial cells at significantly faster rates than RAW117-P cells. Such organ specificity of adhesion was not found at the level of the subendothelial matrix, and the rates of adhesion of RAW117 cells to subendothelial matrix were lower than to endothelial cells. RAW117 cells of low or high metastatic potential bound to immobilized extracellular matrix components, such as fibronectin, at high rates, but adhesion to laminin or collagen IV was minimal. Previous studies indicated that RAW117 lines could proliferate in vitro in certain organ-conditioned media under limiting serum conditions. We therefore examined the ability of a purified paracrine lung growth factor (LDGF-1) to stimulate growth of RAW117 cells in limiting serum-containing medium. The high lung-colonizing L17 line was stimulated to proliferate by LDGF-1 at faster rates than the other lines. The data support Paget's hypothesis that the organ specificity of tumor metastasis is determined by specific tumor cell and host properties.

23/7/13 (Item 13 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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05102701 88011497

Proteins of the rat prostate. II. Synthesis of new proteins in the ventral lobe during castration-induced regression.

Lee C; Sensibar JA

Department of Urology, Northwestern University Medical School, Chicago IL 60611.

Journal of urology (UNITED STATES) Oct 1987, 138 (4) p903-8, ISSN 0022-5347 Journal Code: KC7

Contract/Grant No.: RR05370, RR, NCRR; HD 11611, HD, NICHD

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Ventral prostates from adult Sprague-Dawley rats at different days postcastration were cut into one to two mm .3 pieces and incubated in medium containing S35-methionine (100 uCi/ml.) at 37C under 95% oxygen and 5% carbon dioxide for four hours. The incubated tissues were subjected to two-dimensional electrophoresis and radiofluorography. Over 100 spots were developed in the fluorograms. Three groups of spots, representing cytoskeletal proteins, androgen-dependent proteins and castration-induced proteins, were further evaluated by a computer-based densitometer. The level of densitometry absorption is proportional to the amount of radioactivity in each spot. The synthesis of cytoskeletal proteins, such as actin and tropomyosin, were relatively constant throughout the course of prostatic regression. The rate of synthesis of androgen-dependent proteins declined rapidly from a high level of synthesis before castration to a non-detectable level by Day 3 postcastration. However, three proteins, which were either not synthesized (spot G and spot H) or synthesized at a very low level (spot I) before castration, were the major proteins synthesized by the prostate during early stages of its regression. The rate of synthesis of these proteins reached a peak by Day 4 postcastration, declined rapidly and remained at a low level thereafter. The respective molecular weights and isoelectric points for these three proteins were 33 Kd and 7.2 for spot G, 38 Kd and 5.3 for spot H and 64 Kd and 6.0 for spot I. Previous findings showed that prostatic regression in rats was associated with a surge of activities in proteolytic enzymes which peaked five to six days postcastration. The peak of synthesis of three proteins noted in the present study, therefore, preceded the peak of activities of proteolytic enzymes in the regressing prostate by one to two days. Testosterone replacement to animals at the time of castration prevented the synthesis of these proteins in the prostate. Since the synthesis of these three proteins in the ventral prostate is induced by androgen-depletion resulted from castration, they are considered as the castration-induced proteins.

23/7/14 (Item 14 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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04951563 87008218

A method for isolating large numbers of viable disaggregated cells from various human tissues for cell culture establishment.

Gibson-D'Ambrosio RE; Samuel M; D'Ambrosio SM

In vitro cellular & developmental biology (UNITED STATES) Sep 1986, 22

(9) p529-34, ISSN 0883-8364 Journal Code: HEQ

Contract/Grant No.: ES3101, ES, NIEHS

Languages: ENGLISH

Document type: JOURNAL ARTICLE

A method is described for the isolation of large numbers of viable disaggregated cells from human tissues. This method combined the mechanical action of a Stomacher Model 80 Lab Blender, 0.1 mg/ml trypsin or 0.5 mg/ml collagenase, and 0.1 mM [ethylene bis(oxyethylenenitrolo)]-tetraacetic acid (EGTA). Tissue (0.2 to 1.0 g) obtained from human fetal intestine, kidney, liver, lung, and skin were separately minced into approximately 1-mm³ pieces. The pieces were placed in a sterile bag containing 60 ml of calcium-magnesium-free phosphate buffered saline, the appropriate enzyme (0.1 mg/ml trypsin or 0.5 mg/ml collagenase) plus 0.1 mM EGTA, and 0.1% methylcellulose. The bag was then placed into the blender and mixed at a low speed for 3 to 20 min at room temperature. After a single cell suspension was observed by phase contrast microscopy, 10 ml of bovine calf serum was added to the cell suspension to inactivate the proteolytic enzymes. At this time 130 ml of cold Hanks' balanced salts solution containing 5% bovine calf serum was added and the entire cell suspension passed through a tissue sieve (100 mesh, 140 micron) and the cells collected by centrifugation. These cells were then resuspended into the appropriate culture medium. In comparison to other methods for establishment of cell cultures from human tissues, the method described requires shorter incubation times with relatively low concentrations of proteolytic enzymes, and yields two- to three-fold greater number of cells per tissue with 86 to 93% viability. Also, depending on the cell type, 50 to 75% of the isolated cells attached to the culture vessel within 24 h. Variation of the time and concentration of digestive enzymes can be used to select different cell types for culture.

23/7/15 (Item 15 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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04860638 85201626

Stimulus-permeability coupling in rat pulmonary macrophages challenged by *Pseudomonas aeruginosa*. An X-ray microanalysis study.

Smith NK; Lewinski AK; Mangos JA; Boyd RL

Cell and tissue research (GERMANY, WEST) 1985, 240 (2) p461-5, ISSN 0302-766X Journal Code: CQD

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Electron probe X-ray microanalysis (XRMA) of freeze-dried ultrathin sections provides the capability of measuring intracellular elemental content. This methodology was used to investigate the stimulus-permeability coupling responses associated with phagocytosis of *Pseudomonas aeruginosa* by cultured pulmonary alveolar macrophages (PAMs) of rats. PAMs were challenged with *P. aeruginosa* suspended in Gey's buffer at a bacteria to PAM ratio of 50:1 for 1 h at 37 degrees C. A 1-mm³ pellet of the unchallenged control PAMs, challenged PAMs and *P. aeruginosa* alone was quench-frozen in nitrogen-cooled, liquid propane, and 0.1-micron cryosections were cut at -100 degrees C. X-ray spectra were collected for nucleus and cytoplasm of 39 control PAMs, 36 challenged PAMs and 40 *P. aeruginosa*. Concentrations (mmole/kg dry weight) were obtained for Na, Cl, K, Ca, Mg, P, S for each cell. In the control PAMs, the content was similar

to other mammalian cells. Moreover, there were no differences in elemental content between nucleus and cytoplasm. In the challenged PAMs, Na concentration was 4 times that of control PAMs (p less than 0.001) whereas Cl was double (p less than 0.001), K was 29% lower (p less than 0.001), and Ca was 4 times higher (p less than 0.05). The elemental concentration profile in the *P. aeruginosa* was distinctly different from that of the PAMs: higher Na, Ca, Mg, but lower Cl and K values. These results demonstrated elemental content changes in cultured PAMs challenged with *P. aeruginosa* that indicate a stimulus-permeability response by membranes associated with the phagocytic process.

23/7/16 (Item 16 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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04365812 84079819

[In vitro cultivation of vital tissue slices: a new variation of organ culture technics]

Zur in vitro-Kultivierung vitaler Gewebeschnitte: Eine neue Variation der Organkulturtechnik.

Nissen E; Tanneberger S; Weiss H; Bender E
Biomedica biochimica acta (GERMANY, EAST) 1983, 42 (7-8) p907-16,
ISSN 0232-766X Journal Code: 9YX

Languages: GERMAN Summary Languages: ENGLISH
Document type: JOURNAL ARTICLE ; English Abstract

A new modification of the organ culture technique is described. In contrast to the conventional technique in which organ pieces (1 mm³) are cultivated, uniform, viable, microscopically controlled tissue slices are used in a modified organ culture technique. The new method is suitable for in vitro culture of human mammary carcinoma. Flow cytometry measurements reveal changes prior to and after collagenase treatment of human mammary carcinomas. In organ culture the composition of cell population prior to and after 48 h could be maintained. Examination under the microscope confirmed these results. The uptake of [3H]-thymidine was greater in tumor slices than in tumor pieces. Organ cultures sufficiently represent carcinoma in vivo in comparison to the other mentioned in vitro techniques.

23/7/17 (Item 17 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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04150177 84264862

Assessing tumor drug sensitivity by a new in vitro assay which preserves tumor heterogeneity and subpopulation interactions.

Miller BE; Miller FR; Heppner GH
Journal of cellular physiology. Supplement (UNITED STATES) 1984, 3
p105-16, ISSN 0737-1462 Journal Code: HNC
Contract/Grant No.: CA-27419, CA, NCI; CA-28366, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE

We have designed an in vitro assay to assess the influence of tumor subpopulation interactions on drug response. The assay is based upon inhibition of growth of 1 mm³-pieces of tumor embedded in a collagen gel matrix. Tumor growth is quantitated by planimetry of each colony's

image, formed with a split image tracing device attached to an inverted microscope. That expansion of the colonies in collagen gel represents growth through cell replication was demonstrated by releasing and counting cell nuclei. Outgrowths from pieces of tumors produced by a series of mouse mammary tumor subpopulation lines expanded in collagen gel at a rate characteristic of each cell line: the growth rate of tumor pieces was similar to that of the corresponding tumor line embedded as a cell bolus of cultured cells, indicating that growth of pieces of tumor is due to the tumor cells rather than to stromal components. When two cell lines were grown together in collagen cultures, interactions affecting growth rate were observed. Both tumor pieces and cell boluses from cultured cells of the relatively homogeneous cell lines displayed similar, characteristic sensitivities to adriamycin (ADR) in the collagen gel assay. Advantages of the collagen assay over cloning assays are (1) preservation of potential cellular interactions which may be important in assessing tumor drug sensitivity; (2) maximization of growth of all cell populations within the tumor, as compared to growth in agar; and (3) reflection of the zonal distribution of different subpopulations within tumors; and (4) simulation of the three-dimensional growth architecture found in vivo.

23/7/18 (Item 18 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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04099587 85181955
Invasiveness in vitro of two mammary adenocarcinoma tumors with different metastasizing ability.
Puricelli L; Colombo LL; Bal de Kier Joffe E; de Lustig ES
Invasion & metastasis (SWITZERLAND) 1984, 4 (4) p238-46, ISSN
0251-1789 Journal Code: GV4
Languages: ENGLISH
Document type: JOURNAL ARTICLE

The adhesive and invasive capacities of a transplantable mammary adenocarcinoma that grew spontaneously in a BALB/c mouse with a moderate lung metastatic ability (M3) and a related variant tumor with a higher metastatic potential (MM3) were confronted with precultivated fragments of neonatal syngeneic lung and kidney in three-dimensional culture. M3 cells adhered only to lung as isolated clusters or surrounded the fragments, forming a dense rim. These adherent cells invaded half of the lung pieces in few places and as thick tongues. On the other side, the highly metastatic tumor MM3 adhered to lung and kidney with a similar frequency, mostly surrounding the confronted fragments as a monolayer. MM3 cells deeply infiltrated the lung and kidney pieces, at several points up to 70 and 100%, respectively, either as fine strands or as solitary cells. Invasion by MM3 cells was always accompanied by a dense, homogeneous, acidophilic necrosis of the whole fragments. The higher in vitro invasiveness of MM3 cells could be associated to their higher metastasizing potential in vivo.

23/7/19 (Item 1 from file: 5)
DIALOG(R) File 5:Biosis Previews(R)
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06931226 BIOSIS NO.: 000089064621
PROTEINS OF THE RAT PROSTATE III. EFFECT OF TESTOSTERONE ON PROTEIN

SYNTHESIS BY THE VENTRAL PROSTATE OF CASTRATED RATS
AUTHOR: SENSIBAR J A; ALGER B; TSENG A; BERG L; LEE C
AUTHOR ADDRESS: DEP. UROL., NORTHWESTERN UNIV. SCH. MED., 303 E. CHICAGO
AVE., CHICAGO, ILL. 60611.
JOURNAL: J UROL 143 (1). 1990. 161-166. 1990
FULL JOURNAL NAME: Journal of Urology
CODEN: JOURA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Protein synthetic activities in the ventral prostate were assessed by two-dimensional electrophoresis in either four-day or seven-day castrated rats at different intervals following subcutaneous implantation of testosterone-filled silastic tubings for a period of up to four days. Prostatic tissues were cut into one to two mm .3 pieces and incubated in tissues culture medium containing S35-methionine (100 .mu.Ci/ml.) at 37.degree.C under 95% oxygen and 5% carbon dioxide for four hours. The incubated tissues were subjected to two-dimensional electrophoresis and radiofluorography. Analysis of protein spots detected in the fluorograms by computer-assisted densitometry revealed temporal changes in the synthesis of individual proteins by the ventral prostate of castrated rats following androgen treatment. Changes in two groups of proteins were evaluated: castration-induced proteins and androgen-dependent proteins. The level of synthesis of three castration-induced proteins (spots G, H, and I) declined rapidly upon testosterone treatment and reached a non-detectable level for spots G and H and a low level of synthesis for spot I by three days following androgen treatment. Synthesis of androgen-dependent proteins (spots D, E, and F) was activated by testosterone treatment. However, the time interval required to activate the synthesis of these proteins is different. Synthesis of protein spot D (prostatic binding protein) was detected as soon as half hour after the treatment. Synthesis of spots E and F, on the other hand, was not activated until 24 and 48 hours after the treatment, respectively. These changes in patterns of protein synthesis represent the characteristics of cellular responses to testosterone stimulation by the regressed prostate.

23/7/20 (Item 2 from file: 5)
DIALOG(R) File 5: Biosis Previews(R)
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04339419 BIOSIS NO.: 000078068963
IN-VITRO CULTURE OF VITAL TISSUE SLICES A NEW VARIATION OF THE ORGAN
CULTURE TECHNIQUE
AUTHOR: NISSTN E; TANNEBERGER S; WEISS H; BENDER E
AUTHOR ADDRESS: ZENTRALISNT. KREBSFORSCHUNG, AKADEMIE WISSENSCHAFTEN DDR,
ROBERT-ROESSLE-INST., BEREICH KLIN. EXP. CHEMOTHER. 1115 BERLIN-BUCH,
DDR.
JOURNAL: BIOMED BIOCHIM ACTA 42 (7-8). 1983 (RECD. 1984). 907-916. 1983
FULL JOURNAL NAME: Biomedica Biochimica Acta
CODEN: BBIAD
RECORD TYPE: Abstract
LANGUAGE: GERMAN

ABSTRACT: A new modification of the organ culture technique is described.
In contrast to the conventional technique in which organ pieces (1 mm³

) are cultivated, uniform, viable, microscopically controlled tissue slices are used in a modified organ culture technique. The new method is suitable for in vitro culture of human mammary carcinoma. Flow cytometry measurements reveal changes prior to and after collagenase treatment of human mammary carcinomas. In organ culture the composition of cell population prior to and after 48 h could be maintained. Examination under the microscope confirmed these results. The uptake of [3H]-thymidine was greater in tumor slices than in tumor pieces. Organ cultures sufficiently represent carcinoma in vivo in comparison to the other mentioned in vitro techniques.

23/7/21 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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02140666 BIOSIS NO.: 000063055668
ELECTRO PHYSIOLOGICAL PROPERTIES OF CHICK EMBRYONIC HEARTS GRAFTED AND
ORGAN CULTURED IN-VITRO
AUTHOR: RENAUD J-F; SPERELAKIS N
JOURNAL: J MOL CELL CARDIOL 8 (11). 1976 889-900. 1976
FULL JOURNAL NAME: Journal of Molecular and Cellular Cardiology
CODEN: JMCDA
RECORD TYPE: Abstract

ABSTRACT: Because young (2-3 day old) embryonic chick hearts organ cultured in vitro for 1-2 wk do not gain the fast Na⁺ channels that they normally would during in situ development, instead retaining their high density of tetrodotoxin(TTX)-insensitive slow Na⁺ channels, whether revascularization and blood perfusion allow membrane differentiation to proceed was tested. Young hearts (3 day old) (ventricular portion) and old hearts (16 day) (small pieces of about 1 mm³) were grafted on to the chorioallantoic membrane of a host chick (6 day old) where they were revascularized by the host circulation; for comparison, hearts were also placed into test-tube organ culture. After periods of 1 wk (grafts) and 2 wk (test-tube cultures), the tissues were removed for intracellular microelectrode recordings. The young hearts had electrical activity similar to fresh non-cultured 3 day old hearts, i.e., the cells failed to further differentiate in culture. The cells had low resting potentials and slowly-rising (5-10 V/s) TTX-insensitive action potentials. There was no difference in results between 2 culture methods. The old hearts cultured by either method had electrical activity similar to fresh non-cultured 16 day old hearts, i.e., the cells retained their highly differentiated state. They had high resting potentials and fast-rising action potentials which were completely abolished by TTX. The action potential duration was longer in the grafted hearts (young and old) than in the test-tube hearts. Young or old myocardial cells tend to retain the membrane cation channels that they originally possessed when placed into culture, and the young grafted hearts fail to gain TTX-sensitive fast Na⁺ channels, even though blood perfused.

23/7/22 (Item 1 from file: 351)
DIALOG(R)File 351:Derwent WPI
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009623049

WPI Acc No: 1993-316598/199340

Culture of protoplast without discolouration to give clone cells - in conventional medium in atmos. contg. 0.1-7.0 per cent carbon dioxide

Patent Assignee: HITACHI CHEM CO LTD (HITB)

Number of Countries: 001 Number of Patents: 001

Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week
JP 5227948	A	19930907	JP 9234897	A	19920221	199340 B

Priority Applications (No Type Date): JP 9234897 A 19920221

Patent Details:

Patent No	Kind	Lan Pg	Main IPC	Filing Notes
JP 5227948	A	4	C12N-005/04	

Abstract (Basic): JP 5227948 A

Culture of protoplast of plants in a medium in an atmosphere of 0.1-7.0% of CO₂.

Protoplast of plants are cultured in a conventional medium contg. 0.1-7.0, pref. 2.0-6.0% of CO₂, opt. contg. plant growth hormone, up to 10 power (2) - 10 power (6), pref. 10 power (3)-10 power (5) cells/ml.

USE/ADVANTAGE - Culture of plant protoplast without discolouration to give clone cells . The cells can be cultured to give plants with stems, leaves and roots.

In an example, a leaf of Brassica oleracea var. gemmifera was pasteurised and cut to pieces of 1-8 mm³ . The pieces were cultured in a synthetic medium, filtered and centrifuged to give pure protoplast cells. The cells 2 x 10 power (4)/ml, were cultured in the synthetic medium for 10 days at 25 deg.C and found 64 colonies/Petri dish

Dwg.0/0

Derwent Class: C06; D16; P13

International Patent Class (Main): C12N-005/04

International Patent Class (Additional): A01H-004/00

23/7/23 (Item 2 from file: 351)

DIALOG(R)File 351:Derwent WPI

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002061565

WPI Acc No: 1978-74629A/197842

Culturing human foetal cells - and injectable compsns. contg. human foetal cells for treating heart, kidney, liver, nervous, genital and circulatory diseases

Patent Assignee: AYGUN S T (AYGU-I)

Inventor: AYGUN S T

Number of Countries: 001 Number of Patents: 003

Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week
DE 2715887	A	19781012				197842 B
DE 2759507	A	19790222				197909
DE 2759508	A	19790222				197909

Priority Applications (No Type Date): DE 2715887 A 19770409; DE 2759508 A 19770409

Abstract (Basic): DE 2715887 A

In the prodn. of human fostal cell cultures , (a) virus free human 3-6 month foetal material is cut into small pieces and washed with Hanks soln. pH 7.2; (b) the comminuted material is incubated with 25% trypsin soln. (changed every 15-30 mins.) at 37 degrees C and pH 7.5 for 20-30 mins.; (c) suspended cells are syphoned off and this procedure repeated until all cells are in suspension; (d) the resulting cells are incubated ca 2 days at 37 degrees C without agitation and dead cells are removed; (e) the cultures are incubated 3-4 days without agitation, the nutrient medium being changed every 2-4 days; (f) optionally, cells are separated from the glass walls by incubating 15-30 mins. with 0.2% trypsin soln. at 37 degrees C, and then subjected to a second incubation as in step (e); (g) when only 30% of the cells are still mitotic, the culture is centrifuged; and (h) the resulting cells are suspended in 15% glycerine-contg. phosphate buffer at a concn. of ca 2000 cells/3 mm³ .

Administration i.v. or i.m. in the treatment of heart disease, kidney disease, liver disease, chronic degenerative disease, circulatory disorders, genital disease and nervous disorders, and to counteract the effects of ageing. The foetal cells used must be in an indifferent state. They can be used instead of fresh animal cells in the cell therapy method of Niehans

Derwent Class: B04; D16

International Patent Class (Additional): A61K-035/48; C12B-003/12;
C12K-009/00

23/7/24 (Item 3 from file: 351)
DIALOG(R)File 351:Derwent WPI
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001809531

WPI Acc No: 1977-30510Y/197717

Determination of glytotoxic properties of biological material - simplified by incubation in culture contg. cerebral tissue from embryo or newly born rats, mice or guinea pigs

Patent Assignee: AM USSR POLIO VIRUS (AMPO-R)

Number of Countries: 001 Number of Patents: 001

Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week
SU 511348	A	19760802				197717 B

Priority Applications (No Type Date): SU 2013404 A 19740405

Abstract (Basic): SU 511348 A

Brain pieces (about 0.5-2 mm³) are cultivated in a typical Hens medium contg. nutrients (glucose) and antibiotics (penicillin), in dishes made from an organic glass with millipore filters (Aufs. type). The atmos. is air +5% CO₂. The concn. is 50-10%.

After 17-24 hr. the cerebral tissue pieces are removed from the nutrient medium and fixed fo 3-12 hr. in an alcohol formalin mixt. then transferred into a paraffin or celluloid. Microscopic slices are cut and coloured.

For longer periods of time, the specimens are stored in 80 degrees alcohol. The test gives positive result, if nuclei of the glyalic cells are decomposed whilst neurons, neuroblasts and vascular elements remain intact

Derwent Class: B04; D16

International Patent Class (Additional): C12K-009/00
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